

PATENT ABSTRACTS OF JAPAN

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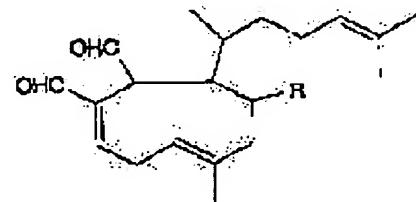
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(54) REVERSE TRANSCRIPTASE INHIBITOR OF HIV-1

(57)Abstract:

PURPOSE: To obtain the subject inhibitor useful as a therapeutic agent and a preventive for AIDS, extremely low toxicity and adverse effects, comprising a specific compound derived from a marine alga as an active ingredient and showing excellent human immunodeficiency virus-1 reverse transcriptase inhibiting activity.

CONSTITUTION: This inhibitor comprises a compound of the formula (R is OH or H) as an active ingredient. The compound of the formula is preferably obtained by extraction from *Dictyota dichotoma* or *Dictyota patens* belonging to the genus *Dictyota* of the family *Dictyotaceae* of blown alga with ethyl acetate, chloroform or acetone. In the case of administering the inhibitor for treating AIDS or its analog diseases, a daily dose is preferably 1-200 μ g/kg (weight) calculated as the compound of the formula per adult.



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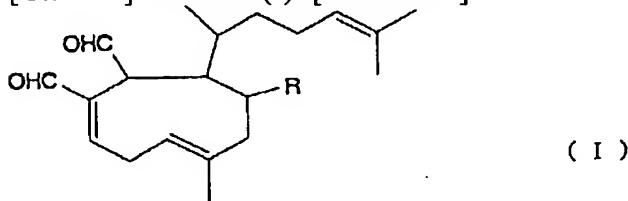
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CLAIMS

[Claim(s)]

[Claim 1] Formula (I): [Formula 1]



It is the reverse transcriptase (it abbreviates to HIV-1 RT) inhibitor of the human immunodeficiency virus -1 which makes an active principle a kind of compound chosen from the compound expressed with (R shows a hydroxyl group or a hydrogen atom among a formula) at least.

[Claim 2] HIV-1 according to claim 1 which makes an active principle the solvent extraction extractives of the seaweed which is chosen from the compound expressed with a formula (I), and which contains a kind at least RT inhibitor.

[Claim 3] Seaweed is fucus Dictyota dichotoma (Dictyotaceae). Department Dictyota dichotoma (Dictyota) HIV-1 according to claim 2 which is seaweed belonging to a group RT inhibitor.

[Claim 4] The seaweed belonging to a fucus Dictyotaceae Dictyota dichotoma group is Dictyota dichotoma (Dictyota dichotoma). Or HIV-1 according to claim 3 which is common AMIJI (Dictyota patens) RT inhibitor.

[Claim 5] HIV-1 according to claim 2 characterized by being a kind of solvent for which the extracting solvent used for solvent extraction is chosen from ethyl acetate, chloroform, an acetone, isopropanol, a pentane, a hexane, a heptane, the ether, and toluene at least RT inhibitor.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Industrial Application] This invention is the inhibitor of the reverse transcriptase (it abbreviates to RT) of the human immunodeficiency virus -1 (it abbreviates to HIV-1) which is a cause virus of acquired immune deficiency syndrome (AIDS), HIV-1 [i.e.,]. It is related with RT inhibitor.

[0002]

[Description of the Prior Art] HIV-1 performs reverse transcription of the self-RNA genome which is a stroke required for a virus duplicate with reverse transcriptase. Therefore, the inhibitor of this reverse transcriptase can serve as anti-HIV matter. Current and azidothymidine (AZT) are used for the therapy of an acquired immunodeficiency syndrome and a similar disease for this purpose. However, the fault of AZT is a side effect by the toxicity, and retrieval of the anti-acquired immunodeficiency syndrome medicine which was more excellent in this way continues. This invention aims at offering the new inhibitor of HIV-1 reverse transcriptase from a natural product.

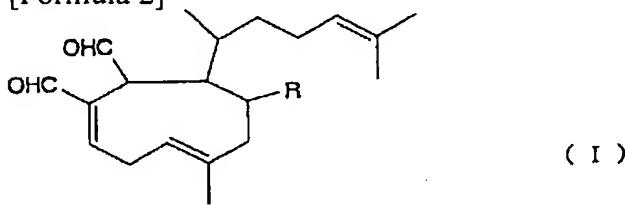
[0003]

[Means for Solving the Problem] this invention persons are HIV-1 with the strong compound which is the matter of the seaweed origin currently used as edible, therefore is expressed with the following formula with very few toxicity and side effects (I). It found out having RT inhibition activity and this invention was completed.

[0004] The summary of this invention is as follows.

** Formula (I) : [0005]

[Formula 2]



[0006] It is the HIV-1RT inhibitor which makes an active principle a kind of compound chosen out of calling it a compound (I) below compound [expressed with (R shows a hydroxyl group or a hydrogen atom among a formula) at least.

** HIV-1 [given in the above-mentioned ** which makes an active principle the solvent extraction extractives of the seaweed which is chosen from a compound (I), and which contains a kind at least] RT inhibitor.

** Seaweed is fucus Dictyota dichotoma (Dictyotaceae). Department Dictyota dichotoma (Dictyota) HIV-1 [given in the above-mentioned ** which is seaweed belonging to a group] RT inhibitor.

** The seaweed belonging to a fucus Dictyotaceae Dictyota dichotoma group is Dictyota dichotoma (Dictyota dichotoma). Or HIV-1 [given in the above-mentioned ** which is common AMIJI (Dictyota patens)] RT inhibitor.

** A HIV-1RT inhibitor given in the above-mentioned ** characterized by being a kind of solvent for which the extracting solvent used for solvent extraction is chosen from ethyl acetate, chloroform, an acetone, isopropanol, a pentane, a hexane, a heptane, the ether, and toluene at least.

[0007] Hydroxy JIKUCHIOJIARU whose R in a formula (I) is a hydroxyl group as a compound (I), and JIKUCHIOJIARU whose R is a hydrogen atom are mentioned about this invention. A compound (I) can be

obtained also by being able to obtain by more nearly usually than the seaweed containing a compound (I), for example, the seaweed belonging to a fucus Dictyotaceae Dictyota dichotoma group, carrying out separation purification, and compounding by the approach of the very thing known. This invention may be presented with a compound (I) as a mode of the solvent extraction extractives from seaweed. As seaweed containing a compound (I), the seaweed which belongs, for example to a fucus Dictyotaceae Dictyota dichotoma group is illustrated, and especially Dictyota dichotoma and Spatoglossum are illustrated as a desirable thing.

[0008] Separation purification of the compound (I) from the seaweed of a fucus Dictyotaceae Dictyota dichotoma group can be performed by extracting with a suitable organic solvent according to a conventional method. seaweed -- desiccation -- even if powdered, it can use also in the state of a raw seaweed object, and it is left in a solvent and a fixed time amount room temperature after stirring, and a solvent extraction object (solvent extraction extractives) can be obtained for the crude extract obtained according to filtration thru/or centrifugal separation vacuum concentration and by hardening by drying. As an extracting solvent, oleophilic solvents, such as hydrophilic solvents, such as a methanol, ethanol, isopropanol, a butanol, and an acetone, and chloroform, ethyl acetate, a pentane, a hexane, a heptane, benzene, toluene, an acetonitrile, and the ether, are desirable. As a desirable extracting solvent, ethyl acetate, chloroform, an acetone, isopropanol, the ether, n-hexane, toluene, etc. are especially mentioned in these. The above-mentioned solvent may be used independently respectively and may be used by plurality.

[0009] Isolation purification and identification of a compound (I) are performed with a conventional method from solvent extraction extractives. For example, a silica gel column chromatography, an ODS column chromatography, high performance chromatography, etc. can separate and refine an extract or its concentration liquid. each fraction -- nuclear magnetic resonance (NMR) -- law and mass analysis (MS) -- it identifies by law etc. It is effective in identification to take further the NMR spectrum of hydrogen (1H) and carbon (13C) for a 2-dimensional shift correlation NMR spectrum etc. about NMR using superconduction Fourier transform (FT) NMR equipment 200MHz or more. Moreover, about MS, field desorption mass analysis (FD-MS) is effective in identification.

[0010] a compound (I) and these solvent extraction extractives -- anti- -- HIV-1 It has RT activity. anti- -- HIV-1 Measurement of RT activity is performed by investigating the amount of incorporation to cDNA of tritiation thymidine mono-phosphate. The inhibitor of this invention checks this incorporation. IC50 added the inhibitor sample to reaction mixture by 0.5-100microg [/ml] concentration, and made it concentration in case the rate of inhibition is 50%. It mixes with independent or a compound and a compound (I) and solvent extraction extractives are pharmaceutical-preparation-ized. Pharmaceutical-preparation-izing, at least one kind of active ingredient, or solvent extraction extractives can be included with a carrier and an excipient permissible on the medicine manufacture beyond one kind or it, and other remedies can be included in arbitration. a carrier -- taking orally -- it passes and the rectum, pernasality, and the carrier suitable for local, the oral cavity, the hypoglottis, vaginal, or parenteral (inside of hypodermically, intramuscular, vein, and hide is included) administration are contained. When using HIV-1 reverse transcriptase inhibitor of this invention for the therapy of an acquired immunodeficiency syndrome and a similar disease, in the case of taking orally, 1-200microg [/kg] (weight) adult 1 sunny is prescribed for the patient in 1 - several steps as a compound (I).

[0011]

[Example] Hereafter, an example and the example of a trial are shown and this invention is explained more concretely.

Fucus Dictyotaceae Dictyota dichotoma extracted on the coast of preparation Ehime Prefecture of example 1 ** solvent extraction extractives (Dictyota) Seaweed Dictyota dichotoma of a group (Dictyota dichotoma) It freeze-dried and ground, after washing with water. 2l. of ethyl acetate was added to 234g of this frost-shattering object, and it stirred with 3 sufficient hours and extracted. While filtering the extract and removing insoluble matter, filtrate was collected and reduced pressure removal of the solvent was carried out. Greenish-brown solvent extraction extractives 2.5g was acquired. A part of these extract extractives were dissolved in dimethylsulfoxide (DMSO), and 10% of DMSO solution was prepared.

[0012] ** Measurement reaction mixture of HIV-1 RT inhibition activity (in 50mM tris-hydrochloric-acid 8.3 60micro buffer-solution pH 1) 1.2microgPoly(rA) and p (dT) 12-18 (template primer; Pharmacia manufacture), 6.0nmoles thymidine triphosphate, 1microcurie tritiation thymidine triphosphate, 10mM magnesium chloride, 50mM potassium chloride, 3mM dithiothreitol, 0.1% Nonidet P-40 (trade name: Nakarai Tesuku make), To the inside containing 10ngHIV(s)-1 reverse transcriptase, the DMSO solution of the solvent extraction extractives prepared by ** is added so that it may become 1%. cDNA(s) generated

after the 1-hour reaction were filtered and collected through the ion-exchange filter paper at 37 degrees C, subsequently it washed by Na₂ HPO₄ and ethanol 5%, and counting was carried out with the liquid scintillation counter. As control, in addition, DMSO was similarly measured so that it might become 1%. Solution extract extractives are HIV-1 at this concentration. RT activity was checked completely.

[0013] In separation / purification example 1 of example 2 ** extract extractives, separation and purification of a compound (I) were performed using solvent extraction extractives 2g extracted with ethyl acetate. That is, it is HIV-1 as a result of an ODS column chromatography (KOSUMO seal 75C18 PUREPPU; column size, a 30mmx500mm; expansion solvent, acetonitrile:water =95:5) and a silica gel column chromatography (MERCK silica gel 60:column size, a 20mmx300mm; expansion solvent, hexane:ethyl acetate = 8:1) rough-refining this extract. The rough purification object A with RT inhibition activity was obtained.

** Fractionation of the isolation rough purification object A of the purification matter A was further carried out with semi preparative isolation high performance chromatography (a column, a YMCPack R&D D-ODS-5 S-5 120A 20mmx250mm; eluent, an acetonitrile acetonitrile:water =60:40 to 100% linear gradient; detector, UV230nm; the rate of flow, 9.0 ml/min), and 0.12g of purification matter A was isolated.

** HIV-1 of the purification matter A Some measurement above-mentioned purification matter A of RT inhibition activity was dissolved in DMSO, and 5% of DMSO solution was prepared. It is HIV-1 completely like an example 1 using this DMSO solution. RT inhibition activity was measured. As control, in addition, DMSO was similarly measured so that it might become 1%. The purification matter A is HIV-1 at this concentration. RT activity was checked completely.

** As a result of adding IC50 purification matter A of the purification matter A to reaction mixture by 0.5-100microg [/ml] concentration and asking for concentration in case the rate of inhibition is 50%, it was 50= 4.3microg [/ml] IC.

** The analysis above-mentioned purification matter A of the structure of the purification matter A was identified, making full use of 270MHz NMR and FD-MS. The main signals of 1 H-NMR, 13 C-NMR, and FD-MS were shown below.

[0014] 1 H-NMR delappm:9.69 (1H, s), 9.33 (1H, s), 7.00 (1H, dd), 5.28 (1H, brd), 4.96 (1H, brt), 4.30 (1H, brt), 1.92 (3H, s), 1.65 (3H, s), 1.51 (3H, s), 1.10 (3H, d)

13 C-NMR delappm:203.9(d), 194.6 (d), 158.2 (d), 148.0 (s), 138.4 (s), 131.4 (s), 124.7 (d), 124.4 (d), 73.7 (d), 52.2 (d), 50.5 (d), 47.9 (t), 39.7 (t), 33.3 (d), 29.6 (t), 25.7 (t), 25.6 (q), 20.3 (q), 17.7 (q), 17.6 (q)

FD-MS(m/z): 318 (M⁺), 161, 109, 69 [0015] From the result of above-mentioned FD-MS, it can check that the purification matter A is matter of molecular weight 318. When it combines with the data of 1 H-NMR and 13 C-NMR, reference [J.Tanaka and T.Higa.Chemistry Letters, 231 -232 page, and 1984], etc. and inquires, it turns out that the purification matter A is hydroxy JIKUCHIOJIARU which is one of the compounds (I).

[0016] In separation / purification example 1 of example 3 ** extract extractives, separation and purification of an active principle were performed using solvent extraction extractives 2g extracted with ethyl acetate. That is, it is HIV-1 as a result of an ODS column chromatography (KOSUMO seal 75C18 PUREPPU; column size, a 30mmx500mm; expansion solvent, acetonitrile:water =95:5) and a silica gel column chromatography (MERCK silica gel column 60:column size, a 20mmx300mm; expansion solvent, hexane:ethyl acetate = 8:1) rough-refining this extract. The rough purification object B with RT inhibition activity was obtained.

** Fractionation of the isolation rough purification object B of the purification matter B was further carried out with semi preparative isolation high performance chromatography (a column, a YMCPack R&D D-ODS-5 S-5 120A 20mmx250mm; eluent, an acetonitrile acetonitrile:water =60:40 to 100% step WAIZU gradient; detector, UV230nm; the rate of flow, 9.0 ml/min), and 0.47g of purification matter B was isolated.

** HIV-1 of the purification matter B Some measurement above-mentioned purification matter B of RT inhibition activity was dissolved in DMSO, and 5% of DMSO solution was prepared. It is HIV-1 completely like an example 1 using this DMSO solution. RT inhibition activity was measured. As control, in addition, DMSO was similarly measured so that it might become 1%. The purification matter B is HIV-1 at this concentration. RT activity was checked completely.

** As a result of adding IC50 purification matter B of the purification matter B to reaction mixture by 0.5-100microg [/ml] concentration and asking for concentration in case the rate of inhibition is 50%, it was 50= 9.2microg [/ml] IC.

** The analysis above-mentioned purification matter B of the structure of the purification matter B was identified, making full use of 270MHz NMR and FD-MS. The main signals were shown below.

[0017] 1 H-NMR delappm:10.19 (1H, d), 9.32 (1H, d), 6.93 (1H, dd), 5.34 (1H, brd), 5.05 (1H, brt), 3.28

(1H, ddd), 1.79 (3H, s), 1.67 (3H, s), 1.59 (3H, s), 0.90 (3H, d)

·¹³ C-NMR deltappm:203.9(d), 194.7 (d), 157.8 (d), 148.8 (s), 138.2 (s), 131.2 (s), 124.6 (d), 122.5 (d), 56.7

(d), 48.8 (d), 41.0 (t), 37.8 (t), 32.9 (d), 29.4 (t), 29.0 (d), 26.0 (t), 25.7 (q), 17.7 (q), 17.5 (q), 17.3 (q)

FD-MS(m/z): 302 (M+), 161, 109, 69 [0018] From the result of above-mentioned FD-MS, it can check that the purification matter B is matter of molecular weight 302. The data of ¹ H-NMR and ¹³ C-NMR, and reference [J. Finer, J.Clardy, and W. Fenical, L.Minale, R.Riccio, J.Battaflle, M.Kirkup and R.E.Moore, J.Org.Chem., and 44 If it combines with a volume, 2044-2047, 1979], etc. and inquires It turns out that the purification matter B is JIKUCHIOJIARU which is one of the compounds (I).

[0019]

[Effect of the Invention] A compound (I) has the outstanding HIV-1 reverse-transcriptase inhibition activity, and is expected to be able to offer the remedy or prophylactic of AIDS from a natural product.

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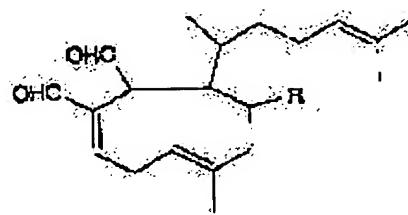
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(54) REVERSE TRANSCRIPTASE INHIBITOR OF HIV-1

(57)Abstract:

PURPOSE: To obtain the subject inhibitor useful as a therapeutic agent and a preventive for AIDS, extremely low toxicity and adverse effects, comprising a specific compound derived from a marine alga as an active ingredient and showing excellent human immunodeficiency virus-1 reverse transcriptase inhibiting activity.

CONSTITUTION: This inhibitor comprises a compound of the formula (R is OH or H) as an active ingredient. The compound of the formula is preferably obtained by extraction from *Dictyota* of *dichotoma* or *Dictyota patens* belonging to the genus *Dictyota* of the family *Dictyotaceae* of blown alga with ethyl acetate, chloroform or acetone. In the case of administering the inhibitor for treating AIDS or its analog diseases, a daily dose is preferably 1-200µg/kg (weight) calculated as the compound of the formula per adult.



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31/11	AED	9454-4C		
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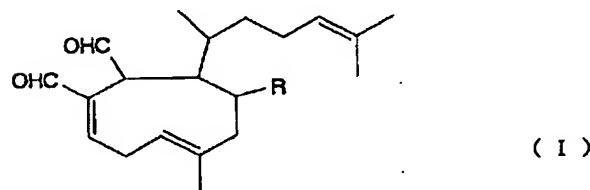
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(54)【発明の名称】 HIV-1 の逆転写酵素阻害剤

(57)【要約】

【構成】 式(1)：

【化1】



(式中、Rは水酸基または水素原子を示す)で表される化合物から選ばれる少なくとも一種の化合物、該化合物含有海藻の溶媒抽出エキスを有効成分とするヒト免疫不全ウィルス-1の逆転写酵素阻害剤。

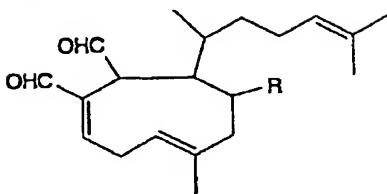
【効果】 該化合物および該溶媒抽出エキスは、優れたヒト免疫不全ウィルス-1の逆転写酵素阻害活性を有し、AIDSの治療薬または予防薬を天然物から提供できると期待される。

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【特許請求の範囲】

【請求項1】 式(I)：

【化1】



(I)

(式中、Rは水酸基または水素原子を示す)で表される化合物から選ばれる少なくとも一種の化合物を有効成分とするヒト免疫不全ウィルス-1の逆転写酵素(HIV-1 RTと略す)阻害剤。

【請求項2】 式(I)で表される化合物から選ばれる少なくとも一種を含有する海藻の溶媒抽出エキスを有効成分とする請求項1記載のHIV-1 RT阻害剤。

【請求項3】 海藻が褐藻アミジグサ(Dictyotaceae)科アミジグサ(Dictyota)属に属する海藻である請求項2記載のHIV-1 RT阻害剤。

【請求項4】 褐藻アミジグサ科アミジグサ属に属する海藻がアミジグサ(Dictyota dichotoma)またはコモンアミジ(Dictyota patens)である請求項3記載のHIV-1 RT阻害剤。

【請求項5】 溶媒抽出に用いる抽出溶媒が酢酸エチル、クロロホルム、アセトン、イソプロパノール、ベンタン、ヘキサン、ヘプタン、エーテルおよびトルエンから選ばれる少なくとも一種の溶媒であることを特徴とする請求項2記載のHIV-1 RT阻害剤。

【発明の詳細な説明】

【0001】

【産業上の利用分野】本発明は後天性免疫不全症候群(AIDS)の原因ウィルスであるヒト免疫不全ウィルス-1(HIV-1と略す)の逆転写酵素(RTと略す)の阻害剤、即ちHIV-1 RT阻害剤に関するものである。

【0002】

【従来の技術・発明が解決しようとする課題】HIV-1は、ウィルス複製に必要な行程である自己RNAゲノムの逆転写を逆転写酵素によって行う。従って、この逆転写酵素の阻害剤は抗HIV物質となり得る。現在、アジドチミジン(AZT)がこの目的でエイズおよび類似疾患の治療に使用されている。しかしながら、AZTの欠点はその毒性による副作用であり、かくしてより優れた抗エイズ薬の探索は続いている。本発明は、HIV-1逆転写酵素の新規な阻害剤を天然物から提供することを目的とするものである。

【0003】

【課題を解決するための手段】本発明者らは、食用として使用されている海藻由来の物質であり、従って極めて毒性・副作用の少ない、下記式(I)で表される化合物

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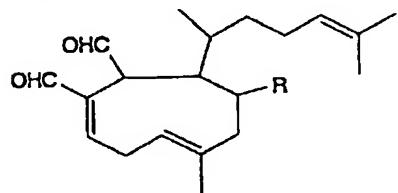
が強いHIV-1 RT阻害活性を有することを見い出し、本発明を完成した。

【0004】本発明の要旨は次の通りである。

① 式(I)：

【0005】

【化2】



(I)

【0006】(式中、Rは水酸基または水素原子を示す)で表される化合物(以下、化合物(I)という)から選ばれる少なくとも一種の化合物を有効成分とするHIV-1 RT阻害剤。

② 化合物(I)から選ばれる少なくとも一種を含有する海藻の溶媒抽出エキスを有効成分とする上記①に記載のHIV-1 RT阻害剤。

20 ③ 海藻が褐藻アミジグサ(Dictyotaceae)科アミジグサ(Dictyota)属に属する海藻である上記②に記載のHIV-1 RT阻害剤。

④ 褐藻アミジグサ科アミジグサ属に属する海藻がアミジグサ(Dictyota dichotoma)あるいはコモンアミジ(Dictyota patens)である上記③に記載のHIV-1 RT阻害剤。

⑤ 溶媒抽出に用いる抽出溶媒が酢酸エチル、クロロホルム、アセトン、イソプロパノール、ベンタン、ヘキサン、ヘプタン、エーテルおよびトルエンから選ばれる少

30 なくとも一種の溶媒であることを特徴とする上記②に記載のHIV-1 RT阻害剤。

【0007】本発明に関して、化合物(I)としては、式(I)におけるRが水酸基であるヒドロキシジクチオジアルと、Rが水素原子であるジクチオジアルが挙げられる。化合物(I)は、通常、化合物(I)を含有する海藻、例えば褐藻アミジグサ科アミジグサ属に属する海藻より分離精製することによって得ることができ、また自体既知の方法にて合成することによっても得ることができる。化合物(I)は、海藻よりの溶媒抽出エキスの

40 様として本発明に供してもよい。化合物(I)を含有する海藻としては、例えば褐藻アミジグサ科アミジグサ属に属する海藻が例示され、特にアミジグサ、コモングサが好ましいものとして例示される。

【0008】褐藻アミジグサ科アミジグサ属の海藻からの化合物(I)の分離精製は、常法に従い適当な有機溶媒で抽出することによって行うことができる。海藻は、乾燥粉末状でも生の海藻体の状態でも用いることができる、溶媒と攪拌後一定時間室温に放置し、濾過ないし遠心分離によって得られた粗抽出液を減圧濃縮、乾固することにより、溶媒抽出物(溶媒抽出エキス)を得ること

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ができる。抽出溶媒としては、メタノール、エタノール、イソブロバノール、ブタノール、アセトン等の親水性溶媒、およびクロロホルム、酢酸エチル、ベンタン、ヘキサン、ヘプタン、ベンゼン、トルエン、アセトニトリル、エーテル等の親油性溶媒が好ましい。これらの中で特に好ましい抽出溶媒としては、酢酸エチル、クロロホルム、アセトン、イソブロバノール、エーテル、n-ヘキサン、トルエン等が挙げられる。上記の溶媒は、各々単独で用いてもよいし、複数で用いてもよい。

【0009】溶媒抽出エキスから化合物(I)の単離精製と同定は、常法にて行われる。例えば、抽出液あるいはその濃縮液をシリカゲルカラムクロマトグラフィー、ODSカラムクロマトグラフィーや高速液体クロマトグラフィーなどで分離・精製できる。各画分を、核磁気共鳴(NMR)法および質量分析(MS)法等により同定を行う。NMRに関しては、200MHz以上の超伝導フーリエ変換(FT)NMR装置を用い、水素(¹H)と炭素(¹³C)のNMRスペクトルを、さらにはシフト相関二次元NMRスペクトル等をとるのが同定には有効である。また、MSに関しては、フィールドテソープション質量分析(FD-MS)が同定には有効である。

【0010】化合物(I)および該溶媒抽出エキスは、抗HIV-1 RT活性を有する。抗HIV-1 RT活性の測定は、トリチウム化チミジンモノホスフェートのcDNAへの取り込み量を調べることで行う。本発明の阻害剤はこの取り込みを阻害する。IC₅₀は、反応液に阻害剤試料を0.5~100μg/mlの濃度で加え、阻害率が50%の時の濃度とした。化合物(I)、溶媒抽出エキスは、単独または配合物と混合して製剤化される。製剤化、少なくとも一種類の活性成分または溶媒抽出エキスを、一種類かそれ以上の製薬上許容できるキャリヤーおよび賦形剤とともに含み、任意に他の治療薬を含むことができる。キャリヤーには、経口、経直腸、経鼻、局所的、口腔、舌下、経膣または非経口的(皮下、筋肉内、静脈内、および皮内を含む)投与に適したキャリヤーが含まれる。本発明のHIV-1逆転写酵素阻害剤をエイズおよび類似疾患の治療用に使用する場合、経口の場合、化合物(I)として、成人1日当たり1~200μg/kg(体重)を1~数回に分けて投与される。

【0011】

【実施例】以下、実施例および試験例を示し、本発明をより具体的に説明する。

実施例1

① 溶媒抽出エキスの調製

愛媛県の沿岸にて採取した褐藻アミジグサ科アミジグサ(Dictyota)属の海藻アミジグサ(Dictyota dichotoma)を水で洗浄した後、凍結乾燥し粉碎した。この凍結粉碎物234gに、酢酸エチル2リットルを加え、3時間よく攪拌して抽出した。抽出液を濾過し、不溶分を除

去するとともに濾液を集め溶媒を減圧除去した。緑褐色の溶媒抽出エキス2.5gを取得した。この抽出エキスの一部をジメチルスルホキサイド(DMSO)に溶解し、10%のDMSO溶液を調製した。

【0012】② HIV-1 RT阻害活性の測定

反応液(50mMトリス-塩酸緩衝液pH8.3 60μl中に、1.2μg Poly(rA)·p(dT)₁₂₋₁₈(テンプレート・プライマー: ファルマシア社製)、6.0nmolesチミジントリホスフェート、1μCiトリチウム化チミジントリホスフェート、10mM塩化マグネシウム、50mM塩化カリウム、3mMジチオトレイトール、0.1%ノニデットP-40(商品名:ナカライテスク(株)社製)、10ng HIV-1逆転写酵素を含む)中に、①で調製した溶媒抽出エキスのDMSO溶液を1%となるように加え、37°Cで1時間反応後生成したcDNAをイオン交換濾紙で濾過して集め、次いで5%Na₂HPO₄、エタノールで洗浄して液体シンチレーションカウンターで計数した。コントロールとして、DMSOを1%となるように加えて同様に測定した。溶液抽出エキスは、この濃度でHIV-1 RT活性を完全に阻害した。

【0013】実施例2

① 抽出エキスの分離・精製

実施例1において、酢酸エチルにて抽出した溶媒抽出エキス2gを用いて化合物(I)の分離・精製を行った。すなわち、この抽出物をODSカラムクロマトグラフィー(コスモシール75C18プレップ:カラムサイズ、30mm×500mm:展開溶媒、アセトニトリル:水=95:5)、シリカゲルカラムクロマトグラフィー(MERCK社シリカゲル60:カラムサイズ、20mm×300mm:展開溶媒、ヘキサン:酢酸エチル=8:1)で粗精製した結果、HIV-1 RT阻害活性のある粗精製物Aが得られた。

② 精製物質Aの単離

粗精製物Aを、更にセミ分取高速液体クロマトグラフィー(カラム、YMC Pack R&D D-ODS-5S-5 120A 20mm×250mm:溶離剤、アセトニトリル:水=60:40からアセトニトリル100%のリニアグラジェント:検出器、UV230nm;流速、9.0ml/min)で分画して精製物質Aを0.12g単離した。

③ 精製物質AのHIV-1 RT阻害活性の測定

上記精製物質Aの一部をDMSOに溶解し、5%のDMSO溶液を調製した。このDMSO溶液を用いて実施例1と全く同様にしてHIV-1 RT阻害活性を測定した。コントロールとして、DMSOを1%となるように加えて同様に測定した。精製物質Aは、この濃度でHIV-1 RT活性を完全に阻害した。

④ 精製物質AのIC₅₀

精製物質Aを反応液に0.5~100μg/mlの濃度

で加え、阻害率が50%の時の濃度を求めた結果、 $IC_{50} = 4.3 \mu\text{g}/\text{mL}$ であった。

⑤ 精製物質Aの構造の解析

上記精製物質Aを270MHzのNMR、およびFD-MSを駆使して同定を行った。 $^1\text{H-NMR}$ 、 $^{13}\text{C-NMR}$ およびFD-MSの主なシグナルを以下に示した。

【0014】 $^1\text{H-NMR}$ δ ppm: 9.69 (1H, s), 9.33 (1H, s), 7.00 (1H, dd), 5.28 (1H, brd), 4.96 (1H, brt), 4.30 (1H, brt), 1.92 (3H, s), 1.65 (3H, s), 1.51 (3H, s), 1.10 (3H, d)

$^{13}\text{C-NMR}$ δ ppm: 203.9 (d), 194.6 (d), 158.2 (d), 148.0 (s), 138.4 (s), 131.4 (s), 124.7 (d), 124.4 (d), 73.7 (d), 52.2 (d), 50.5 (d), 47.9 (t), 39.7 (t), 33.3 (d), 29.6 (t), 25.7 (t), 25.6 (q), 20.3 (q), 17.7 (q), 17.6 (q)

FD-MS (m/z) : 318 (M^+), 161, 109, 69

【0015】上記のFD-MSの結果から、精製物質Aは分子量318の物質であることが確認できる。 $^1\text{H-NMR}$ と $^{13}\text{C-NMR}$ のデータおよび文献 [J. Tanaka and T. Higa, Chemistry Letters, 231~232 頁, 1984 年] 等と併せて検討すると、精製物質Aは化合物(I)の一つであるヒドロキシジクチオジアルであることがわかる。

【0016】実施例3

① 抽出エキスの分離・精製

実施例1において、酢酸エチルにて抽出した溶媒抽出エキス2gを用いて有効成分の分離・精製を行った。すなわち、この抽出物をODSカラムクロマトグラフィー(コスモシール75C18プレップ; カラムサイズ、30mm×500mm; 展開溶媒、アセトニトリル:水=95:5)、シリカゲルカラムクロマトグラフィー(MERCK社シリカゲルカラム60:カラムサイズ、20mm×300mm; 展開溶媒、ヘキサン:酢酸エチル=8:1)で粗精製した結果、HIV-1 RT阻害活性のある粗精製物Bが得られた。

② 精製物質Bの単離

粗精製物Bを、更にセミ分取高速液体クロマトグラフィー(カラム、YMC Pack R&D D-ODS-5S-5 120A 20mm×250mm; 溶離剤、アセトニトリル:水=60:40からアセトニトリル1

00%のステップワイズグラジェント; 検出器、UV 230nm; 流速、9.0ml/min)で分画して精製物質Bを0.47g単離した。

③ 精製物質BのHIV-1 RT阻害活性の測定

上記精製物質Bの一部をDMSOに溶解し、5%のDMSO溶液を調製した。このDMSO溶液を用いて実施例1と全く同様にしてHIV-1 RT阻害活性を測定した。コントロールとして、DMSOを1%となるように加えて同様に測定した。精製物質Bは、この濃度でHIV-1 RT活性を完全に阻害した。

④ 精製物質Bの IC_{50}

精製物質Bを反応液に0.5~100 $\mu\text{g}/\text{mL}$ の濃度で加え、阻害率が50%の時の濃度を求めた結果、 $IC_{50} = 9.2 \mu\text{g}/\text{mL}$ であった。

⑤ 精製物質Bの構造の解析

上記精製物質Bを270MHzのNMR、およびFD-MSを駆使して同定を行った。主なシグナルを以下に示した。

【0017】 $^1\text{H-NMR}$ δ ppm: 10.19 (1H, d), 9.32 (1H, d), 6.93 (1H, dd), 5.34 (1H, brd), 5.05 (1H, brt), 3.28 (1H, ddd), 1.79 (3H, s), 1.67 (3H, s), 1.59 (3H, s), 0.90 (3H, d)

$^{13}\text{C-NMR}$ δ ppm: 203.9 (d), 194.7 (d), 157.8 (d), 148.8 (s), 138.2 (s), 131.2 (s), 124.6 (d), 122.5 (d), 56.7 (d), 48.8 (d), 41.0 (t), 37.8 (t), 32.9 (d), 2

30 9.4 (t), 29.0 (d), 26.0 (t), 25.7 (q), 17.7 (q), 17.5 (q), 17.3 (q)

FD-MS (m/z) : 302 (M^+), 161, 109, 69

【0018】上記のFD-MSの結果から、精製物質Bは分子量302の物質であることが確認できる。 $^1\text{H-NMR}$ と $^{13}\text{C-NMR}$ のデータおよび文献 [J. Finer, J. Clardy, W. Fenical, L. Minale, R. Riccio, J. Battaglia, M. Kirkup and R.E. Moore, J. Org. Chem., 44 卷, 2044~2047, 1979年] 等と併せて検討すると、精製物質Bは化合物(I)の一つであるジクチオジアルであることがわかる。

【0019】

【発明の効果】化合物(I)は、優れたHIV-1 逆転写酵素阻害活性を有し、 AIDSの治療薬または予防薬を天然物から提供できると期待される。

フロントページの続き

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